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Published in:
Physiological Zoology

DOI:
10.1086/physzool.67.2.30163851

Invited Perspectives in Physiological Zoology

Physiologically Inspired Regression Models for Estimating and Predicting Nutrient Stores and Their Composition in Birds

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Accepted 10/27/93

Abstract

Regression models are presented that can be helpful in estimating and predicting the amount and fat and lean composition of nutrient stores in birds, when carcass data on body mass, fat mass, and body size are available. It is assumed that birds will show a breakpoint in composition during starvation, because stores, the nutrients accumulated in anticipation of shortage, have a different composition from that of the structural part of the body. The models differ only in the assumptions on the sources of variation in the data. One model, for example, assumes that all variation is due to unexplained variation in the mass of the structural part of the body. Another model assumes that the only variation is in the composition of the stores. It is shown that these models may yield completely different results. Examples suggest that the most reliable assumption on the source of variation differs among bird species. The need for ancillary information to verify the assumptions is emphasized. Prevailing methods, such as regressing fat mass on body mass and size, or regressing fat mass on condition indices such as body mass divided by size, are shown to be based on questionable assumptions, or to lack any theoretical justification, respectively. Their use should be abandoned.

Introduction

Birds and mammals often face nutrient deficits over periods of days or even weeks. This occurs not only when food is scarce, but also when demands are high and time available for foraging is limited. It occurs on cold and short winter days, during breeding, and during migration. In such periods the amount of stored nutrients is likely to be of vital importance for survival

Physiological Zoology 67(2):305–329. 1994. 0031-935X/94/6702-9319$02.00

or reproduction. It is not surprising, therefore, that many students of birds have tried to find methods to assess the amount of nutrients stored by birds (see Blem [1990] for review). Hitherto many different approaches have been followed. Each has its own, often implicit, objectives and assumptions. Our aim is to make these objectives and assumptions explicit, to show the shortcomings of the methods, and to offer alternatives, when available. We try to achieve this by introducing a general model that takes into account the amount and composition of both the structural part and the stores. The error part of the model is emphasized, because this is an essential step in obtaining estimators and predictors of both the structural part and the stores. Our findings are illustrated with a few real-world examples. For practical reasons there is a strong avian bias in this article, but the problems and solutions should be very similar for other vertebrates.

Body Composition: Structural Part and Stores

We start our discussion by defining (admittedly loosely) stores, reserves, and the structural part of the body (Piersma 1984; Piersma and Davidson 1991). The stores are defined as the nutrients accumulated in anticipation of periods of shortage (King and Murphy 1985). Contrary to stores, reserves are used only in emergencies, because they constitute the tissue necessary for a functionally "normal" life (Lindström and Piersma 1993). In other words, stores are strategically meant to be used as nutrient or energy supply, whereas reserves are not. The structural part of the body consists of those parts that are not stores. Hence it includes the reserves.

Body mass, although often used (Hanson 1962; Owen and Cook 1977; Ankney 1979; Whyte and Bolen 1984; Hobaugh 1985; Hohman and Taylor 1986), is not a good measure of stores, because it does not take into account the mass of the structural part of the body. Body mass minus the mass at death (Piersma 1984; Jenni-Eiermann and Schifferli 1989) is not a reliable measure of stores either, because starving birds can lose a large amount of reserves (which belong to the structural part of the body) before final death. It has the additional disadvantage that the amount of reserves consumed is highly variable and depends on external conditions at the time of death (Vespsalainen 1968; Davidson and Evans 1982; Davidson and Clark 1985). Usually the simplifying assumption is made that stores consist entirely of fat and that lean mass equals the structural part of the body (Odum, Rogers, and Hicks 1964). Recent studies on geese and waders, however, have demonstrated that some species do store both fat and protein (Evans and Smith 1975; Raveling 1979; McLandress and Raveling 1981; Lindström and Piersma 1993). So stores are not equivalent to fat.

A summary of the terminology is given in figure 1*A*. Note that in the literature the term *body* is used ambiguously. Sometimes it refers to the sum of the chemical components (i.e., the sum of water, fat, and fat-free tissue), and sometimes it refers to the sum of the structural part and the stores. We have made no attempts to formalize the use of the word *body*. We think that the meaning will be clear from the context. In this way, we avoid the use of terms like *body mass of the structural part,* or *body mass of the total body*. However, in all equations *body* refers to the sum of the chemical components, while *fat* refers to fat mass and *lean* to lean mass. The subscript *struc* refers to the structural part of the body, the subscript *store* to the stores. If no subscript is used, the sum of the structural part and the stores is referred to. At this point, it should be noted that in all equations uppercase letters denote random variables, lowercase letters denote realizations of random variables (i.e., observed values), and Greek symbols or italics denote parameters.

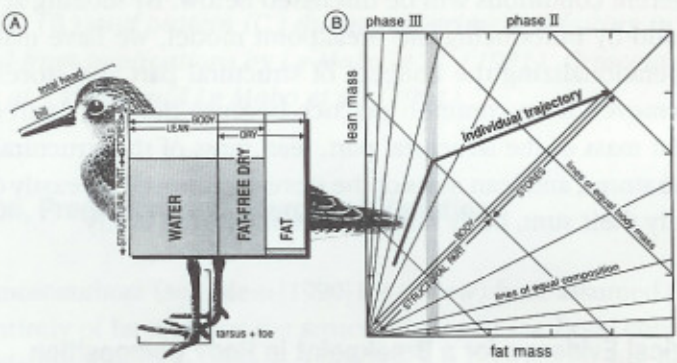

Fig. 1. Basic terminology (A) *and a possible relation between lean mass and fat mass for an individual bird* (B). *The block in bird* A *symbolizes its body and summarizes the two ways of examining composition: either as chemical categories (here, water, fat-free dry, and fat, from left to right) or as structural part (shaded, bottom) and stores (white, top). The terms to describe different combinations of chemical categories are indicated, as are some common measures indicating the size of the structural part (but note that there are several other, more internal ones, available as well). Section* B *gives an example of the change in fat and lean body composition and body mass in a starving individual (thick broken line), with reference to the physiological phases indicated on top of the graph (see text). Lean mass is plotted against fat mass. Lines of equal fat and lean composition and body mass are indicated, as are some of the terms of* A.

The Breakpoint Model

The composition in terms of water, fat, protein, and carbohydrate is likely to differ between the stores and the structural part of the body (fig. 1*A*). This means that, if the body composition of a bird is known over a broad range of body masses, the analysis of the relation between body composition and body mass can reveal both the mass and the composition of structural part and stores. The relationship is expected to show a breakpoint where body mass equals the mass of the structural part. Figure 1*B* shows a possible relationship between fat mass and lean mass, as well as the accompanying relationship between composition (in terms of fat/lean ratio) and body mass. So, for a particular bird at a particular time, two points in figure 1*B* (i.e., its present constitution and its breakpoint) are needed to tell us its structural part and stores (and, possibly, the amount of reserves consumed) at that moment. Each point in figure 1*B* can be represented either in terms of fat and lean mass or in terms of composition (for example, fat/lean ratio) and body mass (sum of fat and lean mass). The preferred representation under different conditions will be discussed below. By looking at the composition, and by introducing the breakpoint model, we have made a step toward operationalizing the concept of structural part and stores. Yet we are still removed from common practice because the four figures that are needed (fat mass of the structural part, lean mass of the structural part, fat mass of the stores, and lean mass of the stores) cannot all be easily obtained. In fact, only their sum, body mass, can be measured easily.

Physiological Evidence for a Breakpoint in Body Composition

A whole body of evidence based on careful studies of the physiological responses to fasting of geese and penguins (Le Maho et al. 1981, 1991; Le Maho 1983; Groscolas 1986; Cherel, Robin, and Le Maho 1988; Boismenu, Gauthier, and Larochelle 1992) indicates a rather sudden and dramatic change in substrate use in the course of fasting, which should correspond to a change in the composition of body mass (fig. 2). After a short period of adaptation (Phase I), fasting birds initially conserve protein (Phase II), up to a point when the fat is almost used up (fig. 1*B*). Then the birds' reliance on lipid catabolism ends and the use of proteins for energy expenditure (and hence blood levels of uric acid and urea, and daily excretion of nitrogen compounds) suddenly increases (Phase III). At body masses larger than the breakpoint, mass changes consist mostly of fat. Below the breakpoint, changes consist predominantly of fat-free tissue (fig. 1*B*).

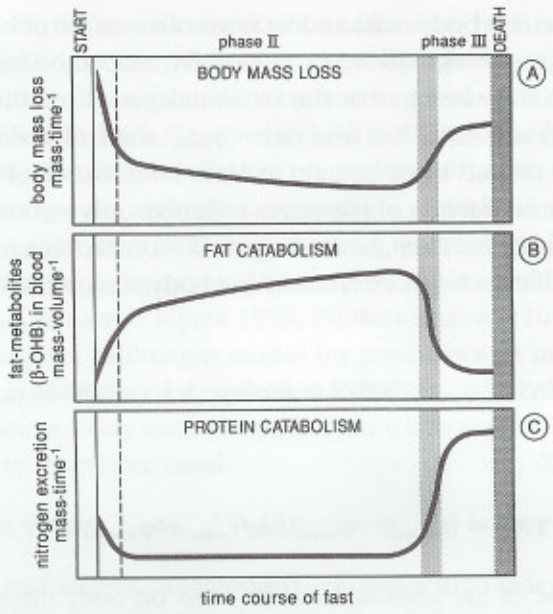

Fig. 2. Summary of the change in body mass (A) and the metabolic reliance on fat (B) and protein (C) during experimental fasting in birds, assembled from publications by Le Maho et al. (1981), Groscolas (1986), Cherel et al. (1988) and Le Maho et al. (1991).

Estimation, Prediction, and Sources of Variation

Hitherto most authors (see Blem [1990] for review) have assumed that stores consist entirely of fat and that the structural part of the body contains only a negligible amount of fat, so their interest has focused on the fat mass of individual birds. Measuring fat mass and subsequent estimation of population parameters are straightforward, but destructive for the birds. Hence methods have been developed that aim to estimate the parameters of a calibration model on a sample of dead birds, in order to enable future prediction of the fat mass of individual live birds given measurements of explanatory variables. However, the equivalence between fat and stores is not straightforward, and both the estimation and the prediction of the mass and the composition of the structural part and the stores is much more complicated. In using calibration models for prediction, dead birds and living birds must be distinguished. For dead birds both body mass and fat mass usually are measured. Hence two more predictions or assumptions (e.g., on fat mass and lean mass at breakpoint) are necessary to complete the description. For living birds only body mass is measured. This means that yet another prediction (or assumption) is needed.

Now suppose that body mass and fat mass of a sample of birds have been measured (by weighing and by extracting fat, respectively) and presume further, for the time being, that the structural part of all birds is of equal mass ($body_{struc}$) and equal fat/lean ratio (φ_{struc}, the fat fraction of the structural part) and that all birds contain at least some stores of equal fat/lean ratio (φ_{store}, the fat fraction of the stores). Hence, only the amount of stores varies between birds. Then, according to the breakpoint model, a linear relationship will exist between fat mass and body mass, which can be written as

$$\text{FAT} = \varphi_{store}(\text{BODY} - body_{struc}) + \varphi_{struc} body_{struc}$$

or, alternatively,

$$\text{FAT} = (\varphi_{struc} - \varphi_{store}) body_{struc} + \varphi_{store}\text{BODY},$$

where the slope of the regression of fat mass on body mass is equivalent to φ_{store}.

Observations will show an inevitable scatter around this line. This scatter might be due to different sources of error, and, if an inappropriate model for the error were applied, the estimator of the slope might be severely biased. We believe that much of the confusion concerning the estimation and prediction of fat mass or nutrient stores in general is due to inappropriate modeling of the error of the models (see Tanner 1949; Atchley, Gaskins, and Anderson 1976; Atchley 1978; Atchley and Anderson 1978; Phillips 1983; Blem 1984, 1990; Reist 1985; Packard and Boardman 1987, 1988; Lindström and Piersma 1993). Preferably, the choice of the error model has to be based on clear and easily understandable assumptions and should result in a simple estimation procedure (see, e.g., Sprent and Dolby 1980; McArdle 1988). Below we will distinguish several alternatives.

Measurement Error

The ordinary least squares regression model assumes a linear relationship between the explanatory variable body mass, measured without error (body = BODY), and the dependent variable fat mass, measured with an error with zero mean and constant variance (fat = FAT + ε):

$$\text{fat} = \alpha + \beta \cdot \text{body} + \varepsilon, \qquad (1a)$$

where, according to the breakpoint model, α is a compound parameter, that is, $(\varphi_{struc} - \varphi_{store}) \cdot body_{struc}$, and $\beta = \varphi_{store}$. Note that, although body mass

contains fat mass, the measurement error of fat mass is not reflected in the measurement of body mass. Most often the simple linear regression model of fat mass on body mass (see, e.g., Iverson and Vohs 1982; Chappell and Titman 1983; Piersma 1984; Gauthier and Bédard 1985; Piersma 1988; Castro and Myers 1990; Carpenter et al. 1993) or multiple regression models of fat mass against body mass and other explanatory variables referring to body size, such as wing length or bill length (see, e.g., Johnson et al. 1985; Perdeck 1985; Ringelman and Szymczak 1985; Sibly, Jones, and Houston 1987; Piersma 1988; Castro and Myers 1990; Piersma and van Brederode 1990) have been used as a calibration model for predicting fat mass. Generally, fat mass has been thought to be equivalent to the mass of the stores. However, in fitting fat mass on body mass and one or more size variables, the following model seems to have been used:

$$\text{fat} = (\varphi_{struc} - \varphi_{store}) \cdot f_1(\text{size}) + \varphi_{store} \cdot \text{body} + \varepsilon, \qquad (1b)$$

which means that several assumptions are implicitly made: (1) the stores do partly consist of nonfat (the fat fraction is reflected in the regression parameter of body mass), (2) the variation in the structural body size is perfectly explained by a function of the size measurements ($body_{struc} = f_1(\text{size})$), and (3) the remaining error is due to measurement error of the fat mass. It is interesting to note that, although emphasis has been put on fat mass, which was believed to indicate the amount of stores, implicitly a model is used that assumes that the stores also do contain nonfat. Besides, we think that assumptions 2 and 3 are very unlikely. Probably most of the remaining error will be due to the inability to explain the variation of the structural body mass in terms of size measurements.

When the measurement error of fat mass is negligible compared to the measurement error of body mass, it is more appropriate to use the reciprocal of the ordinary least squares slope of the regression of body mass on fat mass as an estimator of φ_{store}. When fat mass and body mass are both measured with error, the assumptions of ordinary least squares are no longer valid. Alternatively, the maximum likelihood estimate of the so-called structural relationship model (Sprent 1969; Kendall and Stuart 1973; Sprent and Dolby 1980) can be applied, given that knowledge on the relative magnitude of both error variances is available. The major axis and reduced major axis regressions implicitly assume some ratio of both error variances (see, e.g., McArdle 1988).

Spurious Correlation

It might be argued that the fit of fat mass on body mass just gives the conditional mean of fat mass, given the value of body mass.

The theoretical justification, then, must be found in the unlikely assumption that the bivariate observations (fat, body) are a random sample from a bivariate normal distribution (FAT, BODY). This implies that the conditional mean of FAT, given BODY = body, has the form $\alpha + \beta \cdot$ body, and that the maximum likelihood estimators of the parameters α and β happen to be exactly the same as the ordinary least squares estimators (see, e.g., Sprent and Dolby 1980). Besides, the correlation between fat mass and body mass will be spurious, because body mass is the sum of fat mass and lean mass. Its value r_{fb} depends in a complicated and hardly interpretable way on σ_{fl}, the covariance between fat mass and lean mass, and on σ_f^2 and σ_l^2, the variances of fat mass and lean mass:

$$r_{fb} = \frac{\sigma_f^2 + \sigma_{fl}}{\sqrt{\sigma_f^2 \cdot (\sigma_f^2 + 2\sigma_{fl} + \sigma_l^2)}}.$$

Note that the covariance between fat mass and lean mass might reflect the covariance between stores and structural part (in case fat is equivalent to stores and lean to structural part) or the covariance between the fat and lean parts of the stores (in case the variance in the structural part is negligible). However, the correlation coefficient between fat mass and body mass does not tell us much about the covariance between fat mass and lean mass, and the emphasis that is often put on this correlation coefficient (see, e.g., Blem 1990, p. 71), is unjustified and confusing.

Variation in the Structural Part

When sources of variation other than measurement error are more important, the ordinary least squares regression of fat mass on body mass or vice versa might both be inappropriate. For example, the assumption of equal structural mass could be invalid, and variation in structural mass might form the major source of variation. For convenience, a constant, known structural fat mass is assumed. Hence all variation in the mass of the structural part among birds is due to differences in lean mass of the structural part ($\text{LEAN}_{struc} = lean_{struc} + \varepsilon$). Then, with the additional prerequisites that all birds have at least some stores of equal composition and that no measurement error occurs (body = BODY, fat = FAT, and so lean = LEAN), the appropriate form of the relationship is

$$\text{lean} = lean_{struc} + \frac{1 - \varphi_{store}}{\varphi_{store}} (\text{fat} - fat_{struc}) + \varepsilon. \qquad (2a)$$

In order to remove part of the unexplained variation, body size measurements can be included ($LEAN_{struc} = f_1(size) + \varepsilon$). This leads to

$$lean = f_1(size) + \frac{1 - \varphi_{store}}{\varphi_{store}} (fat - fat_{struc}) + \varepsilon. \qquad (2b)$$

Hence φ_{store} can be easily derived from the ordinary least squares slope of lean mass on fat mass. Ignoring the subtraction of the constant fat_{struc} from the fat mass does not effect the estimation of the slope. The assumption of independent errors with zero mean and constant variance also implies that no relation exists between the total amount of stores and the structural mass.

For dead birds the mass of the lean fraction of the stores can be predicted by $(1 - \varphi_{store})/\varphi_{store} \cdot (fat - fat_{struc})$. This can be seen from $fat_{store} = \varphi_{store} \cdot (fat_{store} + lean_{store})$, and so $(1 - \varphi_{store}) \cdot fat_{store} = \varphi_{store} \cdot lean_{store}$. The fat mass of living birds can be predicted by

$$fat = \varphi_{store}[body - f_1(size)] + (1 - \varphi_{store}) fat_{struc}.$$

For living birds the mass of the lean fraction of the stores can be predicted by

$$lean_{store} = (1 - \varphi_{store})[body - f_1(size) - fat_{struc}].$$

In some cases, one or more other independent calibration functions of lean mass or fat mass are available, for example, obtained by measuring the total body electrical conductivity (tobec) of the bird (Walsberg 1988; Castro, Wunder, and Knopf 1990; Morton, Kirkpatrick, and Smith 1991; Scott, Grant, and Evans 1991),

$$lean = f_2(tobec) + \varepsilon,$$

or by measuring fat score, which is a nondestructive method to assess subdermal fat deposits (see, e.g., Hailman 1985; Rogers 1987; Krementz and Pendleton 1992),

$$fat = f_3(fatscore) + \varepsilon.$$

The total body electrical conductivity device primarily measures water, because the electrical conductivity of water is much higher than the electrical conductivity of fat and fat-free dry mass. This means that only when the water fraction of the fat-free mass is constant can it predict lean mass satisfactorily. At this point it should be noted that the suggestion of Morton et

al. (1991) to apply a multiple regression of fat mass on body mass and electrical conductivity, in order to enable future prediction of fat mass, lacks any sound theoretical justification, and their main argument ("because the variation in lipid mass is primarily reflected in body mass and not lean mass, body mass should be incorporated into the regression model"; Morton et al. 1991, p. 464) is opaque. Their method therefore should be discouraged. Another alternative method of predicting the water content of a living bird is a dilution experiment with water isotopes (Crum, Williams, and Nagy 1985; Forbes 1987). When all these different calibration functions are taken together no unique prediction will be obtained:

$$\text{fat} = \varphi_{store}[\text{body} - f_1(\text{size})] + (1 - \varphi_{store})fat_{struc},$$

$$\text{fat} = \text{body} - f_2(\text{tobec}),$$

$$\text{fat} = f_3(\text{fatscore}).$$

A weighted procedure can be applied to combine the various predictions. The weights could be related to the reciprocal of the variance of the calibration functions.

Until now the mass of the structural body has been calibrated to size variables (McNeil and Cadieux 1972; Bailey 1979; Wishart 1979; Davidson 1983; Piersma 1984; Alisauskas and Ankney 1987; Sibly et al. 1987; Piersma 1988; Jenni-Eiermann and Schifferli 1989) by the following model:

$$\text{lean} = f_1(\text{size}) + \varepsilon.$$

Subsequently fat mass has been predicted by subtracting the predicted mass of the structural body from the measured body mass (McNeil and Cadieux 1972; Davidson 1983). From what was said earlier, it will be clear that this method is also based on the questionable prerequisite that the stores contain only fat, that is, $\varphi_{store} = 1$. Given the possibility that the stores contain nonfat, our model (2b) seems to be more relevant.

Finally, we should mention the unlikely case that all scatter stems from variation in the fat mass of the structural part ($\text{FAT}_{struc} = fat_{struc} + \varepsilon$). Then the relevant model looks like

$$\text{fat} = fat_{struc} + \frac{\varphi_{store}}{1 - \varphi_{store}}(\text{lean} - lean_{struc}) + \varepsilon, \qquad (3)$$

and the fat fraction of the stores can be derived from the ordinary least squares slope of fat mass on lean mass.

Variation in the Composition of the Stores

Yet another model of the error assumes that the only variation concerns the composition of the stores itself. In accordance with Aitchison (1986) and with the assumption that the stores' composition vector ($FAT_{store}/BODY_{store}$, $LEAN_{store}/BODY_{store}$) follows an additive logistic normal distribution, the log ratio $\log(FAT_{store}/LEAN_{store})$ has a normal distribution. Hence, with the assumption of a constant mass and composition of the structural part and with the additional prerequisite that all birds have at least some stores, the following model appears:

$$\log\left(\frac{\text{fat} - fat_{struc}}{\text{lean} - lean_{struc}}\right) = \log\left(\frac{\varphi_{store}}{1 - \varphi_{store}}\right) + \varepsilon. \qquad (4)$$

The model is visualized in figure 3*A*. The constant fat and lean mass of the structural part, fat_{struc} and $lean_{struc}$, have to be assessed a priori. Admittedly, this is a rather arbitrary step. For dead birds predictions of fat_{store} and lean_{store} immediately follow from the assumptions concerning fat_{struc} and $lean_{struc}$. For living birds lean_{store} can be predicted by use of the estimated φ_{store}:

$$\text{lean}_{store} = (1 - \varphi_{store})(\text{body} - fat_{struc} - lean_{struc}).$$

The Breakpoint Included

In all approaches discussed so far, only birds that contained at least some stores were considered. This reduced the number of assumptions needed, but often it is unclear at what point the stores are zero. The last method that will be discussed takes birds from both sides of the breakpoint into account. It assumes that the conditional distribution of the log ratio log(fat/lean), given body mass, is normal. This approach is advocated by Aitchison (1986, p. 222), who discusses the statistical analysis of compositional data. In the ornithological literature the ratio fat/lean is known as the lipid index, and has been used frequently (see, e.g., Bryant and Hails 1983; Rogers, Nolan, and Ketterson 1993). Its untransformed use was criticized by Blem (1984). The expected value of the log ratio in relation to body mass follows a nonlinear relationship (fig. 3*A*), which can be understood by the derivations of equations (1) and (4), given earlier. If body mass is greater than $body_{struc}$ it looks like

$$\log\left(\frac{\text{fat}}{\text{lean}}\right) = \log\left(\frac{(\varphi_{struc} - \varphi_{store}) \cdot body_{struc} + \varphi_{store} \cdot \text{body}}{-(\varphi_{struc} - \varphi_{store}) \cdot body_{struc} + (1 - \varphi_{store}) \cdot \text{body}}\right) + \varepsilon; \qquad (5)$$

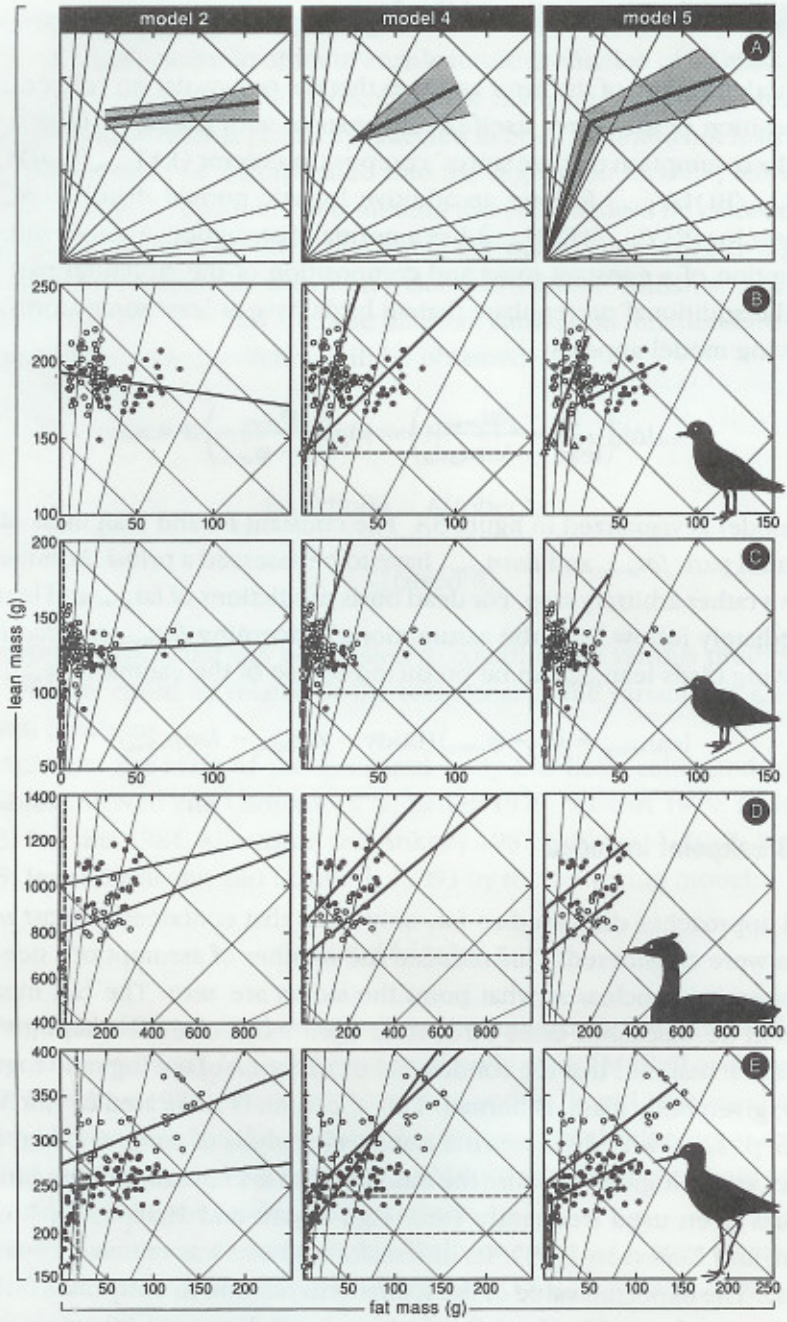

Fig. 3. The three different error structures for the breakpoint model: model (2), model (4), and model (5). A, *Hypothetical relationship, including prediction limits;* B, *greater golden plover;* C, *red knot;* D, *great crested grebe;* E, *bar-tailed godwit. Bird profiles scaled to real size are provided as a reference. For the greater golden plover the open circles*

if body mass is equal to or smaller than $body_{struc}$ it looks like

$$\log\left(\frac{\text{fat}}{\text{lean}}\right) = \log\left(\frac{\varphi_{struc}}{1 - \varphi_{struc}}\right) + \varepsilon. \quad (5)$$

An option for the model is the inclusion of body size measurements, that is, using f_1(size) instead of the parameter $body_{struc}$. Admittedly, the error of model (5) lacks an easily interpretable foundation. It resembles model (4), but the assumption of a constant and known mass and composition of the structural part of the body is loosened. Unfortunately, predictions of all four variables to describe body mass and composition cannot be obtained unequivocally. Parameters were fitted by a simplex minimization algorithm (SYSTAT 5.0; Wilkinson 1990).

Miscellaneous Approaches

One other calibration function can be found quite often in the literature (see Blem 1990): simple linear regression of fat mass on some condition index, for example, the ratio of body mass to body length (see, e.g., Chappell and Titman 1983; Gauthier and Bédard 1985; Briggs 1989). However, the relationship between fat mass on one hand and a ratio of body mass and some size variable on the other hand cannot be derived from any theoretical model (e.g., our breakpoint model). Nor can its use be based on the assumption of a bivariate normal distribution of the variables. The use of condition indices for predictive purposes must therefore be distrusted.

Situations might occur in which no training set of dead birds is available for calibration purposes. Yet several methods have been published that enable the prediction of fat mass without prior use of dead birds (e.g., Ellegren 1989; Ellegren and Fransson 1992). The simplest method that circumvents any measuring of fat mass is based on the assumption that the mass of the structural body is equal for all individuals. So body mass predicts the fat

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represent birds caught in spring, the filled circles birds caught in winter, and the triangles experimentally starved spring birds. For the other three species open circles indicate females, and filled circles, males. For the great crested grebe and the bar-tailed godwit all models were fitted for the sexes separately. For model (2) the dashed line shows the threshold in fat mass. Data below this threshold were deleted from the model estimation procedure. For model (4) the intersection of the horizontal and dashed vertical line indicates the mass of the structural body.

mass apart from a constant. Even if the structural part is equal for all individuals a disadvantage is that only absolute differences in fat mass are predicted: it makes quite a difference whether two birds have fat masses of 10 and 34 g or of 56 and 80 g. A refinement of this method uses birds with a fat score of zero, assuming that these birds do not contain any stores, to calibrate the mass of the structural body to size measurements. So calibration takes place without direct fat measurements. A still further refinement assumes a linear relationship between fat mass and fat scores and uses all birds available to estimate the following relationship (Ellegren and Fransson 1992):

$$\text{body} = f_1(\text{size}) + f_2(\text{fatscore}) + \varepsilon.$$

Subsequently fat mass is predicted by

$$\text{fat} = \text{body} - f_1(\text{size}).$$

This model therefore assumes that lean mass can be predicted perfectly by size measurements. All error is explained by the imperfect correlation between fat score and fat. Unfortunately, the data themselves are able neither to support nor to reject this assumption, and the accuracy of the prediction remains unclear. However, in those cases where measured lean mass was related to body size measurements, the correlation was far from perfect and r^2 was usually below 0.6 (McNeil and Cadieux 1972; Bailey 1979; Davidson 1983; Alisauskas and Ankney 1987; Sibly et al. 1987; Jenni-Eiermann and Schifferli 1989).

An Artificial Example

Piersma and Jukema (1990) performed a reduced major axis regression of lean mass on body mass to arrive at an estimate of the fat fraction of the stores. They implicitly assumed that the error variance of each variable was proportional to its sample variance. This assumption seems rather unrealistic (Sprent and Dolby 1980), and Piersma and Jukema (1990) did not give any compelling arguments in favor of its use. Lindström and Piersma (1993) showed that this reduced major axis regression of lean mass on body mass can yield severely biased results. They achieved this by constructing an artificial sample, based on the following model:

$$\text{FAT} = (0.3 + \delta)(body_{struc} + \varepsilon),$$

where δ and ε are errors according to some specified probability model. We use these data to illustrate that the models presented previously can yield widely differing estimates for the fat fraction of the stores.

For these artificial data the assumption of equal size of the structural part is not met and, furthermore, a dependence exists between the amount of stores (here $\text{BODY}_{store} = \text{FAT}$) and the size of the structural part ($body_{struc} + \varepsilon$). Evidently, neither the reduced major axis method of Piersma and Jukema (1990), nor any of the models presented so far in this article will be able to reproduce precisely that the stores entirely consist of fat (table 1). It should be realized that many possible sources of error exist, and that all methods take only one or a few into account. This means that, for each method (with its underlying assumptions on the error), artificial examples (using another assumption) can be constructed quite easily to show the inability of the method in coping with data with a different error model. Therefore, the construction of imaginary examples to illustrate the limitations of a certain method is of limited value. More important is an awareness of the underlying assumptions of each method and a recognition that additional information on the true error sources is needed to decide on the most suitable method.

For all models mentioned above, except model (5), table 1 gives the estimate of the fat fraction of the stores for Lindström and Piersma's (1993) hypothetical example of 33 male bar-tailed godwits (*Limosa lapponica*) and for an empirical data example of 48 male bar-tailed godwits. The empirical data set is the same as the one used by Lindström and Piersma (1993). Table 1 shows that the estimated fat fraction strongly depends on the assumed error. Even when we leave out the ordinary least squares regression of fat mass on lean mass, which is based on the unlikely assumption that the error stems from variation in the amount of fat in the structural part and which yields the lowest fat fractions for both cases, the range of estimated values is still large: 0.68–0.96 for the hypothetical godwits and 0.25–0.91 for the real ones. It is interesting, but not surprising, to see that the ordinary least squares regression of lean mass on fat mass, which is based on the idea that the scatter is due to variation of the structural part, reveals a value of 0.96 for the hypothetical godwits, which is rather close to the imposed value of one. The remaining difference is due to the imposed dependence of the amount of stores on the size of the structural part in the hypothetical godwits. The ordinary least squares regression of body mass on fat mass, although based on another starting point, gives similar results.

TABLE 1

Estimated fat fraction of the stores of hypothetical male bar-tailed godwits and empirical male bar-tailed godwits

Model	Type	Dependent Variable	Explanatory Variable	Hypothetical Godwit ($n = 33$)	Empirical Godwit ($n = 48$)
1a	OLS	fat	body	.87	.64
	OLS	body	fat	.96	.91
2a	OLS	lean	fat	.96	.91
3	OLS	fat	lean	.23	.17
4	OLS	$\log\left(\frac{\text{fat} - fat_{struc}}{\text{lean} - lean_{struc}}\right)$		.88	.25
	RMA	fat	body	.91	.77
	MA	fat	body	.91	.73
	RMA	lean	body	.68	.46

Note. Hypothetical data are from Lindström and Piersma (1993); empirical data are for the same sample used by Lindström and Piersma (1993). The same systematic model was applied, but different error was assumed. OLS, Ordinary least squares; RMA, reduced major axis; MA, major axis. For the log ratio estimator the constant $lean_{struc}$ was set to 200 g and the constant fat_{struc} to 0 g.

Empirical Examples

Data are used from the greater golden plover, *Pluvialis apricaria,* the red knot, *Calidris canutus,* the great crested grebe, *Podiceps cristatus,* and the bar-tailed godwit, *Limosa lapponica.* These species show an increasing extent of sexual size dimorphism. The data stem from a variety of sources, and because most have been used in previous publications we refer to these for a description of their origin and the laboratory practicalities (greater golden plover: Jukema and Piersma 1992; red knot: Piersma, Koolhaas, and Dekinga 1993; great crested grebe: Piersma 1984; bar-tailed godwit: Piersma and Jukema 1990).

Results for model (2) are given in table 2. Data from birds that might have utilized some of their reserves had to be removed. This was achieved by deleting all birds with a fat mass below some specified threshold. By choosing a rather large threshold the risk that starved birds are included is minimized without introducing a bias into the estimate of the composition of the stores, φ_{store}. For the plover all data were used, except those for a few experimentally starved birds.

TABLE 2

The fat fraction of the stores estimated by model (2)

	Greater Golden Plover	Red Knot	Great Crested Grebe	Bar-tailed Godwit
Threshold	...	1.5	25	18
Mean	188.4	127.8	964.5	276.9
SD	13.2	12.0	119.3	35.9
Without size:				
n	91	96	48	78
φ_{store}	>1	.97	.80	.68
rse	13.1	12.0	118.8	31.9
r	.79	.80	.58	.75
External size:				
Variable	*wht*	*wht*	*lwk*	*wbt*
n	83	89	48	74
φ_{store}	>1	.97	>1	.79
rse	12.3	11.4	61.1	21.1
r	.80	.83	.81	.86
Internal size:				
Variable	$\sqrt{abcdef}$	$\sqrt{abcdef}$	...	*abc*
n	90	62	...	78
φ_{store}	>1	>1	...	.81
rse	11.7	10.9	...	23.6
r	.82	.87	...	.84
Sex:				
n	91	90	48	78
φ_{store}	>1	.95	.79	.77
rse	12.9	11.8	76.4	24.0
r	.80	.82	.74	.83

Note. Observations with a fat mass below the threshold value (g) were deleted; "mean," mean of the lean mass (g); SD, standard deviation of the lean mass; rse, square root of the residual mean square; r, correlation between measured and predicted amount of fat; "variable," product of size measures, where w = wing, h = total head, t = tarsus plus toe, b = bill, l = body length, k = keel, a–f = several sternum measures (see Piersma, Davidson, and Evans [1984] and Jukema and Piersma [1992] for definitions).

For the knot, plover, and godwit several external size measures, such as the length of the wing, bill, total head, and tarsus plus toe, and several internal sternum measures were taken. The latter measures were included in separate regressions, because they can only be taken from dead birds. For the grebe only external measures (body, keel, and wing length) were available. Stepwise procedures were used to identify a set of possible models to be considered. The first principal component of the correlation matrix of the explanatory variables was also considered (Alisauskas and Ankney 1987). Furthermore, products of several combinations of three length measures, and even the square root of the product of the six sternum measures, were taken into account. By taking the square root the dimension of the product of the six sternum measures turns into a volume. This results in a regression coefficient that indicates a (scaled) density, that is, mass · length^{-3}. Generally, a single product of three length variables explained approximately the same variance as the linear combination of the separate corresponding variables. The variance explained by the first principal component was also of the same order of magnitude. For convenience, only the results of the best-fitting product are reported. Figure 3 shows the results of the simplest version of the model, in which no size measurements were taken into account.

For the red knot and the greater golden plover all models explained only a small part of the total variance in lean mass. Table 2 shows that the square root of the residual mean square (sometimes called the standard error of the estimate) was only slightly smaller than the standard deviation of the lean mass. For the knot the estimated regression coefficient of fat was never significantly different from zero, implying that the fat fraction of the stores was close to one. For the plover, all models yielded a negative regression coefficient for fat. Although the coefficient was not significant in any case, it does not fit with the ideas underlying the model. For the other two species, the bar-tailed godwit and the great crested grebe, the model explained a much larger part of the variance. External size and fat explained about 68%–75% of the total variance. Sexual dimorphism was, to a large extent, accounted for by size. The regression coefficients for size were always significantly different from zero in both species. The coefficient for fat was never significantly different from zero for the grebe, but it always was for the godwit. For both species, the coefficient for fat was not significantly different between the sexes.

Table 3 shows the results for model (4). For the godwit and the grebe the assumption of constant structural mass was made for each sex separately. Figure 3 clearly shows that the use of model (4) results in a large estimated variance of the composition of the stores for all species. The composition ranges from almost completely fat towards entirely lean. At first sight this

seems unlikely. However, when the plover data set is studied in more detail, a remarkable difference arises between birds caught in spring and birds caught in autumn (fig. 3*B*). Spring birds have a lower fat mass and a higher lean mass. Size differences between both samples, which might explain the larger lean mass of spring birds, could not be detected. Indeed, Jukema and Piersma (1992) show that the species is extremely monomorphic. Besides, banding recoveries confirm that the birds from both samples belong to the same biological population (J. Jukema, personal communication). When model (4) is fitted for the spring and winter samples separately, the estimate of φ_{store} equals 0.25 for the spring birds and 0.51 for the winter birds. Five spring birds were experimentally starved before being killed. Figure 3 suggests that, indeed, these birds have lost predominantly nonfat during starvation. The difference in composition of the stores between both samples also explains the undesirable negative regression coefficient for fat in model (2). It seems that model (4) gives a much better description of the main features of the plover data than model (2).

Table 4 shows the results of model (5). Predicted values differ from those of model (2) but are more or less comparable to those of model (4) (see also fig. 3). Conditional on body mass (the diagonals from upper left to lower right indicate constant body mass), the log ratio log(fat/lean) is normally distributed. This means that, for a large body mass, the differences between the predictions and the observations are strongly down weighted on the original scale for fat and lean mass. This is an attractive property if the scatter stems mainly from variation in composition of the stores (as is assumed in model [4]). However, it is an undesirable feature when most of the variation originates from differences in $lean_{struc}$. Figure 3 shows that, for

TABLE 3

The fat fraction of the stores estimated by model (4)

	Greater Golden Plover	Red Knot	Great Crested Grebe		Bar-tailed Godwit	
			Male	Female	Male	Female
n	91	96	24	24	61	30
$lean_{struc}$	140	100	850	700	200	240
φ_{store}	.32	.24	.48	.55	.42	.51

Note. Observations with a lean mass below the assumed $lean_{struc}$ (g) were deleted; φ_{struc} was set to 0.015.

the knot, model (4) and model (5) yield mostly positive residuals for a body mass between the breakpoint and about 150 g. For a body mass larger than 150 g most residuals do have a negative value. This suggests that the models (4) and (5) give an inappropriate description of the knot data. Figure 3 shows that a similar pattern can be found for the godwit and the grebe.

Summary and Conclusions

The possible existence of many different sources of error prevents a single and general approach to the problem of estimating and predicting the size and composition, in terms of fat and lean, of nutrient stores in birds. However, it seems likely that some sources of variation are less important than others. Presumably variation due to measurement error of body mass and fat mass is low. The same is true for variation in the composition of the structural part of the body. On the contrary, variation in the structural mass and variation in the composition of the stores may be large. When only variation in the composition of the stores is large, as seems to be true for the greater golden plover, model (4) may be used for estimation and prediction. When the variation in the structural mass is large, the use of model (2) is more appropriate.

Contrary to models (2) and (4), model (5) enables the estimation of the breakpoint. Unfortunately the model lacks interpretable assumptions underlying the error. Hence the usefulness of the model is questionable.

We are not aware that the models (2) and (4) have ever been used before in the avian storage literature. Emphasis has most often been put on model (1), where fat mass is regressed on body mass, often in combination with

TABLE 4

The mass of the structural body φ_{struc}, *and* φ_{store} *estimated by model (5)*

	Greater Golden Plover	Red Knot	Great Crested Grebe		Bar-tailed Godwit	
			Male	Female	Male	Female
n	96	96	26	29	66	32
$body_{struc}$	188	117	912	716	246	258
φ_{struc}	.044	.015	.006	.013	.039	.013
φ_{store}	.62	.39	.56	.56	.67	.56

size variables (see, e.g., Iverson and Vohs 1982; Chappell and Titman 1983; Piersma 1984; Gauthier and Bédard 1985; Johnson et al. 1985; Perdeck 1985; Ringelman and Szymczak 1985; Sibly et al. 1987; Piersma 1988; Blem 1990; Castro and Myers 1990; Piersma and van Brederode 1990; Carpenter et al. 1993). Alternatively, the ratio of body mass to size variables has been used as an explanatory variable (see, e.g., Chappell and Titman 1983; Gauthier and Bédard 1985; Briggs 1989). As was discussed earlier, the first approach is based on questionable assumptions and the second method lacks any theoretical ground. Both approaches should therefore be discouraged.

Although analysis of the residuals might be helpful, the assumptions of model (2) (all variation is due to unexplained differences in the structural mass; there is no relation between this variation and fat mass) and model (4) (all variation is due to differences in the composition of the stores) cannot be verified from data on body mass, fat mass, and size indicators alone. Ancillary information is needed. The plover data (seasonal differences, indications of the trajectory in the lean-mass–fat-mass space of starving birds) are an example. Another example is given by Lindström and Piersma (1993), who use the time before departure of a migrating bird population, which is assumed to be synchronized in its fattening and departure schedule, as an explanatory variable.

The linearity assumptions in the breakpoint model, which among other things imply that the fat and nonfat components of the stores are used (during fasting) and stored (during refueling) at a constant ratio, should also be examined in more detail. One counterexample is given by rats that during prolonged fasting lost fat and fat-free tissue at a constant ratio but, when refed, first increased organ and muscle mass and only then redeposited their fat stores (Cherel and Le Maho 1991).

Future research should emphasize the verification of the assumptions discussed in the present article and study the sources of variation in the size and composition of the structural body and the nutrient stores. Whenever the size and composition of nutrient stores are analyzed, the assumptions underlying the employed regression model need to be discussed.

Acknowledgments

We thank Åke Lindström and Nick Davidson for significant contributions to discussions on the description and interpretation of body composition in birds. Many people were practically involved at some stage. We especially thank the members of the Dutch shorebird expeditions to northwest Africa and J. Jukema, P. Duiven, M. R. van Eerden, A. Eleveld, G. A. Gudmundsson, R. Harding, P. L. Meininger, J. Muller, G. Nehls, C. Ott, H.-U. Rösner, A.

Scheele, I. Tulp, and P. M. Zegers for their help. Dick Visser illustrated this contribution, which was improved through the comments of Popko Wiersma, Cajo J. F. ter Braak, and three anonymous referees.

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